MOUSE CAGE DENSITY POLICY

Office of Animal Care and Use
Training and Compliance
University of North Carolina - Chapel Hill
iacuc@med.unc.edu 919-966-5569
UNC Cage Density of Mouse Cages Policy

• The UNC-CH Mouse Cage Density Policy is available here for reference. *Previously referred to as Mouse Breeding Policy.
Mouse Cages at UNC-Chapel Hill

- Static Microisolator Cages
  70 square inches

- Tecniplast Green Line Cages
  78 square inches

- Tecniplast Blue Line Cages
  82 square inches
Cage Density Policy Essentials

For cages with **single litters**:

- No limit on pups with 1 adult
- No limit on pups with 2 adults
- No limit on pups with 3 adults
Cage Density Policy Essentials

For cages with **multiple litters**: no limit on the number of pups, until one litter reaches 14 days old, then:

- 14 pups with 1 adult
- 11 pups with 2 adults
- 9 pups with 3 adults
Cage Density Policy Essentials

<table>
<thead>
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* no need to limit the number of pups until pups reach 14 days of age
Weaning Essentials

The policy requires pups to be weaned at 10 GRAMS, (approximately 21-28 days old)
**Weaning Essentials**

Weaned pup cage density is based on body weight

<table>
<thead>
<tr>
<th>Weight</th>
<th>Floor area per animal</th>
<th>Tecniplast # per cage 78-82 sq in</th>
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“E”xceptions

Necessary deviations will be considered by the IACUC on a case-by-case basis with scientific justification and documentation provided by the PI.

The exception will not be in effect until the PI has received written notification from the IACUC.
“E”xception Examples

Example #1: Lines with larger than average litters
“E”xception Examples

Example #2: Small mouse pup phenotypes
Example #3: Pups with phenotype that makes them appear more mature than they are
Investigator Responsibilities

- Nesting material **MUST** be placed in all breeding cages at the time of setup and until pups are weaned.
Investigator Responsibilities

- Record **Date of Birth** for each litter on all breeding cages. This **must** be recorded within 3 days after birth of the litter.
Investigator Responsibilities

- Write legibly so all information is understandable
Cage Identification – RFID cards

- All cages must be identified by the PI’s name and protocol number. This is accomplished by the use of a DLAM RFID scan card.
No RFID Card?

- If a DLAM RFID card is **NOT** available, the cage still must be labeled with the PI name, Protocol Number and Date Cage Cards Requested can use a plain white card.
# Breeding Records

## Animal Room Breeding Record

<table>
<thead>
<tr>
<th>PI:</th>
<th>Species:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Building:</td>
<td>Room #:</td>
</tr>
<tr>
<td>Year:</td>
<td>Month:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date (1st, 2nd, 3rd)</th>
<th>Protocol #</th>
<th>DOB</th>
<th>Rack #</th>
<th>Cage #</th>
<th># Litters in cage</th>
<th># Pups in cage</th>
<th>Strain</th>
<th>Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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Breeding Etiquette

» Avoid placing more than one male in with females, this leads to potential fighting

» Place food on floor prior to female giving birth

» Remove dead pups from cages once identified
Breeding Etiquette

- Complete genotyping early (so results are ready prior to wean date) to prevent overcrowded cages.
How to age young mice
Be As Accurate As Possible

<table>
<thead>
<tr>
<th>DAY</th>
<th>EVENT</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin real red; short curly whisk; umbilical cord</td>
<td>8</td>
<td>Fuzz on belly</td>
</tr>
<tr>
<td>2</td>
<td>Straight, fine whisk; skin lighter red</td>
<td>9</td>
<td>Fur back; last day belly translucent, milk bands</td>
</tr>
<tr>
<td>3</td>
<td>Ear “nub” prominent</td>
<td>10</td>
<td>Top teeth erupted; belly slight fuzz</td>
</tr>
<tr>
<td>4</td>
<td>Pink smoother skin; ear flaps ½ up, down</td>
<td>11</td>
<td>Fully furred; fur short &amp; smooth</td>
</tr>
<tr>
<td>5</td>
<td>Ears up; white fuzz across shoulders</td>
<td>12</td>
<td>Nipples visible</td>
</tr>
<tr>
<td>6</td>
<td>Bottom teeth erupted</td>
<td>13</td>
<td>Eyes open</td>
</tr>
<tr>
<td>7</td>
<td>Entire back slightly furred</td>
<td>14</td>
<td>Ears open</td>
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Pup Development
Day 1 through Day 25

https://www.youtube.com/watch?v=Nt1HP0-ltF4

IACUC UNC-CH
Do I Have A Male Or Female?

**Males**
- Greater anogenital distance
- Reach sexual maturity at 8 weeks
- Breed up to one year

**Females**
- Reach maturity at 7-8 weeks
- Breed for approximately 6-8 months

IACUC UNC-CH
Do I Have A Male Or Female?

**Neonatal Mice**

- Differentiated by the presence of a **black dot** in male as pinkies

- Once furred females will have teats present

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IACUC UNC-CH

July 2016
WHAT DOES THE YELLOW "Action Required" CARD MEAN?

[Image of a yellow card with guidelines for cage density requirements for laboratory animals]
WHAT DOES THE YELLOW "Action Required" CARD MEAN?

• The cage exceeds UNC’s Cage Density Policy

• The lab has 48 hours (two consecutive days) to address the cage issue

• DLAM will take action to bring the cage into compliance the morning of the 3rd day if not remedied by the lab

• Charges are incurred when DLAM separates the cages
The IACUC expects that these cards will be used as a backup system and that labs will not wait for cards to be placed before handling cages/weaning litters.

Research staff should ensure that minimal cards are placed on cages by properly maintaining cage densities.

Excessive cards are reported to the IACUC.
What’s The Issue?

While checking your colony you come across a cage with 10 adult mice in it.

The cage has too many adults
What's The Issue?

While checking your cages you notice one with multiple litters containing 15 pups and 2 adults. The oldest pups are 15 days old.

There are more pups than the policy allows for multiple litters at day 14.
What’s The Issue?

While checking your cages you notice 9 weanlings in a cage with an Action Required Card on the cage.

They all weigh 15 grams – only 6 should be in the cage.

**Note: If an excessively overcrowded cage is observed by DLAM, they have the authority to take immediate action by separating overcrowded cages.**
“Action Required” CARD IS PLACED ON THE CAGE……..

Cage density must be addressed by research staff within two calendar days

On Monday: Comply by Wednesday
On Tuesday: Comply by Thursday
On Wednesday: Comply by Friday
On Thursday: Comply by Saturday
On Friday: Comply by Sunday

IACUC UNC-CH

July 2016
After Correcting the Cage Density:

A) Invert the Action Required card

B) Throw the Action Required card away

C) Add the Action Required card to your private collection
If there is space:

- If no action is taken by the morning of the 3rd day, then DLAM is required to separate the cage and the lab will be charged.

If there is no space:

- Complete a euthanasia form
- Euthanize only the number of animals that will bring the cage into compliance (starting with the oldest pups)
Research Staff Should Know:

• Repeated or ongoing problems w/ policy will be reported to the IACUC

• Contact IACUC if you cannot comply w/ policy

• As a mouse breeder your lab is responsible for the management of your colony
Research Staff Should Know:

- DLAM is not required to contact you prior to marking or separating your cages so you should check at least every 3rd day!

- The lab will incur a $50 separation fee for every cage separated by DLAM.
Non-Compliance Is Expensive!!

DLAM supervisors will email information from Action Required card to DLAM accounting staff and lab will be billed
Your Lab Didn’t Wean….Now What?

(This is after the 2 day period and there’s no space to separate animals)
DLAM finds 7 adult mice in a cage that are over 25 grams, how many should be euthanized?

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2 ADULT MICE WILL HAVE TO BE EUTHANIZED

Maximum # of animals allowed in a Techniplast cage= 5

IACUC UNC-CH
DLAM finds 2 adult mice (30g each) and 10 pups between 10-15 grams in a cage, how many should be euthanized?

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DLAM finds 2 adult mice (30g each) and 10 pups between 10-15 grams in a Tecniplast (green line) cage, how many should be euthanized?

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4 PUPS WILL HAVE TO BE EUTHANIZED
DLAM finds 3 adults with 3 litters in a Tecniplast cage, what pups need to be euthanized?

- 8 (pinkies)
- 4 (18 day olds)
- 5 (14 day olds)
DLAM finds 3 adults and 3 litters in a Tecniplast cage, what needs to be euthanized?

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The 4 (18 day olds) will have to be euthanized, and 4 of the 5 (14 day olds) will have to be euthanized.
Euthanasia: Rodent Pups and Adults

- The method of euthanasia of pups and adults must be described in the application.

- Ensure only methods approved in the application are used.
Euthanasia: Rodent Pups and Adults

- Unweaned pups that are euthanized prior to weaning do not count towards the total animal number

- Unweaned pups are vertebrate animals and must be treated humanely and in compliance with federal and institutional policies
Network of Laboratory Animal Coordinators

- A useful collection of people that exchange ideas and experiences. (*listserv*)
- Serves as the liaison that improves communication between PIs and the IACUC.
- [http://research.unc.edu/Offices/NLAC/index.htm](http://research.unc.edu/Offices/NLAC/index.htm)
Colony Management Lecture

- The Office of Animal Care and Use conducts voluntary Mouse Colony Management lectures on an *as needed basis*.

- If interested, please contact:
  - Office of Animal Care and Use: 919-966-5569 or email iacuc@med.unc.edu
ANY QUESTIONS, THOUGHTS OR CONCERNS?